

AMENDMENT

It is respectfully requested that the application be amended, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel, as follows:

IN THE SPECIFICATION:

Pages 37, line 23, please rewrite the paragraph thereat as follows:

Plasmid constructions. Standard molecular biology procedures were used (Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular Cloning. A Laboratory Manual (Cold Spring Harbor Press, N.Y.)). The schematic organization of the plasmids used in this study is represented Figure 2. *Gp31* gene was PCR (polymerase chain reaction) amplified using two oligonucleotides 5'-C TTC AGA CAT ATG TCT GAA GTA CAA CAG CTA CC-3' (SEQ ID NO: 1) and 5' -TAA CGG CCG TTA CTT ATA AAG ACA CGG AAT AGC-3' (SEQ ID NO: 2) producing a 358bp DNA using pSV25 (van der Vies, S., Gatenby, A. & Georgopoulos, C. (1994) Nature 368, 654-656) as template. The DNA sequence of a part of the mobile loop of *Gp31* (residues 25 to 43) was removed by PCR, as described (Hemsley, A., Arnheim, N., Toney, M.D., Cortopassi, G. & Galas, D. J. (1989) Nucleic Acids Res. 17, 6545-6551), using oligonucleotides 5' -**GGA** GAA GTT CCT GAA CTG-3' (SEQ ID NO: 3) and 5'-**GGA** TCC GGC TTG TGC AGG TTC-3' (SEQ ID NO: 4), creating a unique *BamH* I site (bold characters). *GroEL* gene minichaperone (corresponding to the apical domain of *GroEL*, residues 191 to 376; (Zahn, R., Buckle, A.M., Peret, S., Johnson, C. M. J., Corrales, F. J., Golbik, r. & Fersht, A. R. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 15024-15029)) was amplified by PCR using oligonucleotides, containing a *BAMH* I site (underlined), 5'-TTC GGA TCC GAA GGT ATG CAG TTC GAC C- 3' (SEQ ID NO: 5) and 5'-GTT GGA TCC AAC GCC GCC TGC CAG TTT C- 3' (SEQ ID NO: 6) and cloned into the unique *BamH* I site of pRSETA-*Gp31* Δ loop sequence. The single ring *GroEL*_{SRI} mutant contains four amino acid substitutions (R452E, E461A, S463A, and V464A) into the equatorial interface of *GroEL*, which prevent the formation of double rings (Weissman, J. S., Hohl, C. M., Kovalenko, O., Kashi, Y., Chen, S., Braig, K., Saibil, H.R., Fenton, W.A. & Horwich, A.L. (1995) Cel 83, 577-587). The corresponding mutations were introduced into *groEL* by PCR (Hemsley, A., Arnheim, N., Toney, M.D., Cortopassi, G. & Galas, D. J. (1989) Nucleic Acid Res. 17, 6545-6551) using

oligonucleotides 5'-TGA GTA CGA TCT GTT CCA GCG GAG CTT CC' (SEQ ID NO: 7) and 5' -ATT GCG GCG AAG CGC CGG CTG CTG TTG CTA ACA CCG-3' (SEQ ID NO: 8) and pRSETA-*Eag* I GroEL or GroESL vectors (Chatellier, J., Hill, F., Lund, P. & Fersht, A.R. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 9861-9866) as template; silent mutations, in respect to the codon usage in *E. coli*, create a unique *Mfe* I (bold characters) and *Nae* I (underlined). *GroEL* (E191G; *groEL44* allele) gene wa PCR amplified form *E. coli* SV2 strain (Zeilstra-Ryalls, J., Fayet, O., Baird, L. & Georgeopoulos, C. (1993) J. Bacteriol. 175, 1134-1143) using two oligonucleotides 5'-T AGC TGC CAT ATG GCA GCT AAA GAC GTA AAA TTC GG-3' (SEQ ID NO: 9) and 5'-ATGTAA CGG CCG TTA CAT CAT GCC GCC CATGCC ACC-3' (SEQ ID NO: 10) producing a 1,659 bp DNA with unique sites for *Nde* I and *Eag* I (underlined). The different genes were subcloned into the unique *Nde* I and *Eag* I unique sites of pACYC184, pJC and pBAD30 (Guzman, L.-M., Belin, D., Carson, M.J. & Beckwith, J. (1995) J. Bacteriol. 177, 4121-4130) vectors (Chatellier, J., Hill, F., Lund, P. & Fersht, A.R. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 9861-9866). A colony-based PCR procedure was used to identify the positive clones (Chatellier, J., Mazza, A., Brousseau, R. & Vernet, T. (1995) Analyt. Biochem. 229, 282-290). PCR cycle sequencing using flourescent dideoxy chain terminators (Applied Biosystems) were performed and analysed on an Applied Biosystmes 373A Automated DNA. All PCR amplified DNA fragments were sequenced after cloning.

Page 53, line 14, please rewrite the paragraph thereat as follows:

Plasmid constructions Standard molecular biology procedures were used (Sambrook et al., 1989). The plasmid pRSETA encoding GroES gene as been described (Chatellier et al. 1998. 1998 In vivo activities of GroEL minichaperones. Proc. Natl. Acad. Sci. USA 95, 9861-9866). The GroES mutant Gly24Trp was generated by polymerase chain reaction (PCR), as described (Helmsley et al., 1989 A simple method for site directed mutagenesis using the polymerase chain reaction. Nucl. Acids Res. 17, 6545-65510 using the template pRSETA encoding GroES (Chatellier et al., 1998) and the oligonucleotides 5' -C GGC TGG ATC GTT CTG ACC G-3' (SEQ ID NO: 11) and 5' -GC AGA TTT AGT TTC AAC TTC TTT ACG-3' (SEQ ID NO: 12), creating a *Nae* I site (Bold characters).

Page 54, line 9, please rewrite the paragraph thereat as follows:

The DNA sequence encoding a part of the mobile loop of GroES (residues 16 to 33) was removed by PCR, as described (Hemsley et al., 1989), using the oligonucleotides 5' - TCC GGC

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TCT GCA GCG G-3' (SEQ ID NO: 13) and 5' – TCC AGA GCC AGT TTC AAC TTC TTT
ACG C –3'(SEQ ID NO: 14), creating a unique BamH I site (bold characters) and the vector
pRSET A-Gro ESΔloop.

After page 57 and before the first page of claims (58), please insert the enclosed papers
titled --sequence listing.--